

## The Claims

What is claimed is:

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1. A method for the purification and recovery of encysted protozoa, comprising separating the encysted protozoa from a suspension comprising the encysted protozoa by a salt flotation process wherein the salt comprises sulfates, phosphates, nitrates, acetates of ammonium, sodium, potassium, calcium, magnesium, or zinc, hydrogen-bonded organics, the salts of guanidine, or mixtures thereof, or a gas flotation process.
  2. The method of claim 1, wherein separating the encysted protozoa is accomplished by a sodium sulphate flotation process which comprises:
    - preparing an admixture comprising the encysted protozoa and the sodium sulphate;
    - centrifuging the slurry and recovering a supernatant therefrom;
    - forming a dilution of the supernatant and centrifuging the dilution; and
    - recovering the concentrate from the centrifuged dilution.
  3. The mixture of claim 2, further comprising:
    - homogenizing the admixture by high intensity homogenization.
  4. The method of claim 2, wherein the sodium sulphate is present in the admixture in an amount from about 3 to about 30 weight percent.
  5. The method of claim 2, wherein the specific gravity of the dilution is less than the specific gravity of the encysted protozoa.
  6. The method of claim 2, wherein the concentrate comprises from about  $1 \times 10^4$  to about  $1.5 \times 10^6$  encysted protozoa/ml.
  7. The method of claim 1, wherein separating the encysted protozoa is accomplished by the gas flotation process which comprises:
    - adjusting the suspension to a pH sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa;
    - conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent compound to selectively coat particles in the suspension and a sufficient amount of a heteropolar compound to produce a stable froth;
    - passing the conditioned suspension through at least one gas flotation cell; and
    - recovering the encysted protozoa from the gas flotation cell.

8. The method of claim 7, wherein the gas is air.

9. The method of claim 8, wherein the suspension pH is about 2.5 to about 3.5.

5 10. The method of claim 7, wherein the surface active agent compound comprises a sodium salt of long-chain alkyl hydrogen sulfate, a quaternary ammonium compound, a blend of a fatty ammonium acetate and 2-ethylhexanol, an ester/amide compound, an alkyloxy polyethylenoxyethanol, or mixtures thereof.

10 11. The method of claim 7, wherein the heteropolar compound comprises amyl alcohols, butyl alcohols, terpinols, cresols, or mixtures thereof.

12. The method of claim 7, wherein the gas flotation cell has a gas rate from about 0.25 to about 1.1 volumes of gas per volume of suspension per minute.

15 13. The method of claim 7 wherein the gas flotation cell comprises at least two serial gas flotation units.

14. The method of claim 13, wherein the at least two units comprise different gas flow rates.

20 15. A method for the sporulation of oocysts, comprising:  
forming an aqueous suspension of the oocysts with water and hydrogen peroxide, wherein the hydrogen peroxide is present in an amount sufficient to eliminate unwanted microbiological growth; and

25 aerating the aqueous suspension to sporulate the oocysts.

16. The method of claim 15, wherein the aqueous suspension is aerated for a time period greater than about 40 hours such that the aqueous suspension during aeration has a dissolved oxygen level greater than about 80% of the saturation level at a temperature of about 22°C to about 32°C and  
30 with an agitation level sufficient to adequately suspend all the solids.

17. The method of claim 15 wherein the aqueous suspension comprises an oocyst concentration of about  $10^4$  to about  $10^6$  oocysts/ml and an initial hydrogen peroxide concentration of about 1,000 to about 20,000 mg/l.

35 18. A method for the purification, recovery, and sporulation of oocysts, comprising:  
separating the oocysts from a first suspension comprising the oocysts; and  
sporulating the separated oocysts by the method of claim 14.

19. The method of claim 18 wherein the oocysts are *Eimeria maxima*, *Eimeria mitis*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria praecox*, or combinations thereof.

20. The method of claim 18 wherein separating the oocysts is accomplished by a sodium sulfate flotation process which comprises:

preparing an admixture comprising the oocysts and the sodium sulphate;  
centrifuging the slurry and recovering a supernatant therefrom;  
forming a dilution of the supernatant and centrifuging the dilution; and  
recovering the concentrate from the centrifuged dilution.

21. The method of claim 18 wherein separating the oocysts is accomplished by a gas flotation process which comprises:

adjusting the first suspension to a pH of sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa;

conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent compound to selectively coat particles in the suspension and a sufficient amount of a heteropolar compound to produce a stable froth;

passing the conditioned suspension through at least one gas flotation cell; and  
recovering the encysted protozoa from the gas flotation cell.

22. The method of claim 21, further comprising:

adding a bleaching agent to the sporulated oocysts in an amount sufficient to inactivate residual microorganisms and eliminate residual organic matter; and  
bleaching the sporulated oocysts.

23. The method of claim 22, wherein the bleaching is conducted concurrently with the sporulation, and the bleaching agent is hydrogen peroxide.

24. The method of claim 22, wherein the bleaching agent is sodium hypochlorite present in an amount from about 5,000 to about 10,000 parts per million free available chlorine, ozone present in an amount up to about 3% in air, or combinations thereof.

25. The method of claim 22, further comprising washing the bleached oocysts by cross-flow membrane filtration to decrease the residual bleaching agent concentration to an acceptable level.

26. The method of claim 25, wherein the bleaching agent is sodium hypochlorite present after washing in a concentration sufficient to suppress residual microbial growth, and the bleached and washed oocyst suspension has a concentration from about  $1 \times 10^6$  to about  $2.5 \times 10^6$  oocysts/ml, a

maximum solids size of less than about 200 microns, and a sodium sulphate content of less than about 0.9 percent.

27. The method of claim 26 further comprising:

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                  combining sterile concentrates of one or more species of oocysts into a combined  
concentrate; and  
                  packaging the combined concentrate under sterile conditions.

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